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Scientific Article

Morphological analyses of otoliths indicate a single population of invasive *Arapaima gigas* in tributaries of the Upper Madeira River (Amazon Basin)

Marcos de Almeida Mereles[1], Raniere Garcez Costa Sousa[2]*, Carlos Edwar de Carvalho Freitas[1]

[1]Universidade Federal do Amazonas – Manaus (AM), Brazil.
[2]Universidade Federal de Rondônia – Porto Velho (RO), Brazil.
***Corresponding author:** ranieregarcez@unir.br

ABSTRACT

The present study aimed to evaluate the potential of the shape of the sagitta otolith to discriminate populations of *Arapaima gigas* outside their natural area of occurrence in different tributaries of the Madeira River. Otolith samples were collected at three locations along the Guaporé and Mamoré rivers. The shape of the otolith was described using 20 Fourier harmonics and five shape indices. No significant differences were found in the shape of the otoliths among the different sample areas, and no evidence of relevant differences between groups was observed graphically for the two methods used. The success of the global classification of the models was considered average, being 57.57% for the shape indices, and 55.54% for the Fourier coefficients. Thus, the results suggest a single population of pirarucu in different locations of the Guaporé and Mamoré rivers. However, multidisciplinary studies involving genetics, morphology, and ecology may provide more accurate guidance for determining pirarucu populations in the study region.

Keywords: Fourier analysis; Shape indices; Fish populations; Sagitta.

Análises morfológicas em otólitos indicam uma única população invasora de *Arapaima gigas* em tributários do Alto Rio Madeira (Bacia Amazônica)

RESUMO

O presente estudo teve como objetivo avaliar o potencial da forma do otólito *sagitta* para discriminar populações de *Arapaima gigas* fora da sua área de ocorrência natural, em diferentes afluentes do Rio Madeira. Amostras de otólitos foram coletadas em três localidades ao longo dos rios Guaporé e Mamoré. A forma do otólito foi descrita usando 20 harmônicos de Fourier e cinco índices de forma. Não foram encontradas diferenças significativas no formato dos otólitos entre as diferentes áreas amostrais. Tampouco foram evidenciadas diferenças entre os grupos quando observados graficamente os modelos de análise discriminantes. O sucesso da classificação global dos modelos foi mediano, sendo 57,57% para os índices de formas e 55,54% para os coeficientes de Fourier. Dessa forma, os resultados sugerem uma única população de pirarucu em diferentes localidades dos rios Guaporé e Mamoré, no entanto estudos multidisciplinares envolvendo genética, morfologia e ecologia podem fornecer orientação mais precisa para a determinação das populações de pirarucu na região de estudo.

Palavras-Chave: Análise de Fourier; Índices de forma; Populações pesqueiras; *Sagitta*.

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INTRODUCTION

Arapaima gigas (Schinz, 1822), popularly known as pirarucu in Brazil, is one of the largest freshwater scaled fish in the world and can reach up to 3 m in length and over 240 kg (Imbiriba et al., 1985). It presents parental care, obligatory air breathing, relatively late sexual maturity, and a preferably piscivorous feeding habit, though it can be opportunistic and takes advantage of other resources available in the environment (Castello et al., 2013a). Endemic to the Amazon basin, it occurs naturally in the sub-basins of the Amazon, Tocantins-Araguaia and Essequibo rivers, which cover Brazil, Ecuador, Colombia, Guyana, and Peru (Castello and Stewart, 2010). It inhabits mainly lentic environments, such as floodplain lakes, and places with waterfalls, and strong currents are potential barriers to its dispersion, thus limiting the geographical distribution of the species (Castello et al., 2013b). However, due to large floods, disruption of natural barriers and the introduction of the species via fish farms, the occurrence of the pirarucu has been recorded in aquatic systems outside its native area (Miranda-Chumacero et al., 2012; Carvalho et al., 2015; Casimiro et al., 2018; Sousa et al., 2022).

In the Madeira River basin, the pirarucu occurs naturally in areas located downstream of the extinct Teotônio waterfall (Doria et al., 2020). However, in the 70s, the species was introduced into tributaries in the Alto Madre de Dios basin with the aim of repopulating the environments considered favorable to its establishment (Carvajal-Vallejos et al., 2017). After its introduction, the species expanded its distribution and is nowadays found in the Beni and Mamoré basins (Lizarro et al., 2017; Carvajal-Vallejos et al., 2017). More recently, with the advent of the construction of the Santo Antônio hydroelectric dam, the natural obstacle that prevented the dispersion of these animals to upstream locations was effectively removed, thus allowing this species to invade areas where they did not previously occur (Doria et al., 2020). Another factor that facilitates the dispersion of this species is the numerous fish farms distributed in the vicinity of streams in the state of Rondônia (IDARON, 2017; Sousa et al., 2022), whose tanks frequently overflow or break due to the seasonal hydrological pulses that are common in the Amazon region (Miranda-Chumacero et al., 2012). This enables the pirarucu to escape to these new environments, as occurred in the Guaporé Valley (A Tribuna, 1977).

Currently, there are records that show the wide distribution of pirarucu in the main tributaries of the upper Madeira River (Sousa et al., 2022), which has aroused the concern of government agencies and professional fishers, since little is known about the damage that these specimens can cause to local fish assemblages (Doria et al., 2020). In this sense, to create appropriate management measures for the species and ensure the integrity of the ecosystem, more studies are needed that show the distribution pattern of pirarucu populations introduced into the tributaries of the Madeira River (Catâneo et al., 2022).

The discrimination of fish stocks using the morphological, structural and chemical properties in otoliths has been extensively studied by fisheries scientists in recent decades in various environments (Bird et al., 1986; Ré, 1994; Sousa et al., 2016; D'Iglio et al., 2021; Mereles et al., 2021). Otoliths are important structures in fish and record natural information at different time scales, providing data such as age and growth, movement patterns, and habitat interactions (Campana, 1999; Begg et al., 2005). The morphological characteristics in turn are specific to each species (L'Abée-Lund, 1988) and may vary geographically within the same species due to environmental and genetic factors (Cardinale et al., 2004; Stransky et al., 2008), thus allowing the identification of populations (Mereles et al., 2021). In addition, the analysis of the shape of the otoliths is considered an inexpensive way to study populations and can provide new insights into conservation and management measures (Tuset et al., 2008).

Several methods have been used to describe and compare the shape of otoliths in morphological studies, including linear dimension ratios (Yedier, 2021), biorthogonal grids (Wang et al., 2010), thin-plate splines (Lombarte et al., 2010), shape indices (Tuset et al., 2003), and several variations of Fourier analysis (Chen et al., 2000). Fourier elliptic functions represent an accurate method for describing and characterizing contours, and efficiently capture contour information in a quantifiable manner (Kuhl and Giardina, 1982; Lestrel, 1997). On the other hand, the shape indices (circularity, roundness, rectangularity, form factor, and ellipticity) are easy to calculate and provide accurate answers in order to discriminate different populations (Tuset et al., 2003).

In this context, defining a more precise methodology to understand the distribution and origin of *A. gigas* populations in new areas of occurrence is crucial for the development of specific management strategies for the control of this invasive species (Catâneo et al., 2022). In the search for less costly and more accurate alternatives to clarify the geographical distribution of *A. gigas* specimens in the tributaries of the upper Madeira River, the present study aimed to investigate whether variation occurs in the shape of the sagitta otolith of pirarucu specimens that could indicate different populations among collection sites. Therefore, we understand that knowledge about the dispersal patterns and colonization of new areas by invasive fish populations and the identification of their invasion routes and vectors constitute important information to the development of fishing management strategies.

MATERIALS AND METHODS

Study area

The study was conducted in three locations in the tributaries of the Guaporé and Mamoré rivers on the upper portion of the Madeira River basin (Rondônia state, Brazil): Corte das Mercedes, on the upper Mamoré River (Fig. 1a); Resex of the Cautário River, on the lower Guaporé River (Fig. 1b); and the biological reserve of the Middle Guaporé River (Fig. 1c). These regions correspond to areas where *A. gigas* does not occur naturally (Sousa et al., 2022).

Data collection

The collections were carried out in October 2018 (Corte das Mercedes, Mamoré River) and in September and October 2020 (Resex of the Cautário River and ReBio of the Guaporé River, respectively). The specimens were caught via experimental fisheries involving local fishers, under license Nº. 65,059-1 from the Chico Mendes Institute for Biodiversity Conservation. Groups of gill nets, made with braided silk yarn number 108, 29 cm between opposite knots, 2.5-m high and 20-m long, were used in the fisheries. These nets remained into the water for 24 hours.

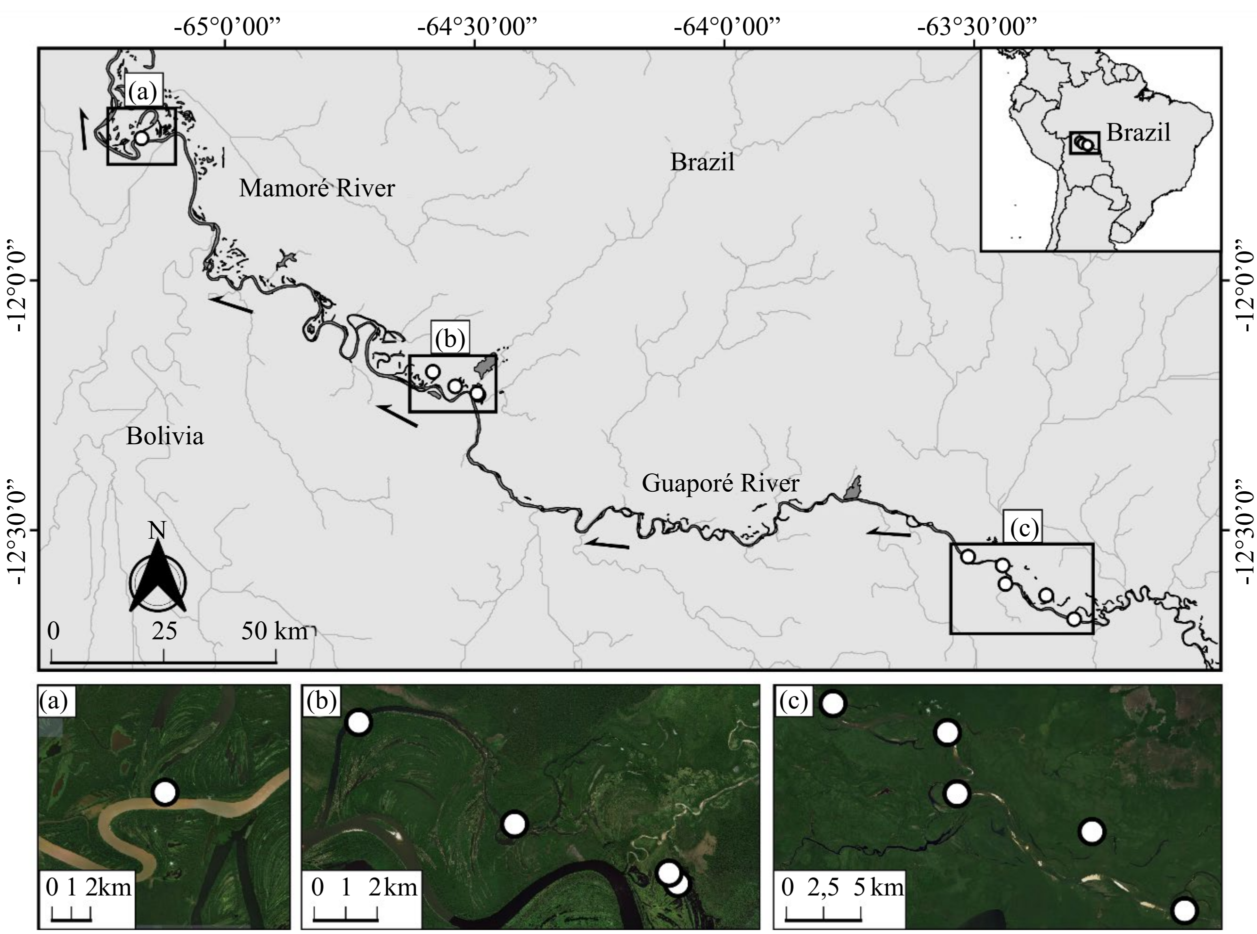

Figure 1. Location of collection points for pirarucu on the Guaporé and Mamoré Rivers. (a) Corte das Mercedes, upper Mamoré River; (b) Extractive Reserve of the Cautário River, lower Guaporé River; (c) Biological Reserve of the middle Guaporé River. The white dots correspond to the collection sites.

During the fish collections, longlines with size 10 or 12 hooks were also used. These devices were randomly installed throughout the collection areas in open areas and near the riverbanks and were checked every 2 hours. The collected specimens were euthanized by stunning via cerebral concussion followed by cranial perforation, which causes disruption of the functioning of vital organs quickly and irreversibly (CONCEA, 2013).

Subsequently, measurements of total length (cm) and total weight (g) were taken using a tape measure and a set of scales (with a precision error of 0.001 g). The skulls of the animals were opened while still in the field to obtain sagitta otoliths for analysis. Only the otoliths from the right side were used for morphometric analysis.

Acquisition of images

Two-dimensional digital images of the right otoliths of each specimen were captured using a USB digital camera (Olympus, SC30) at 10x magnification coupled to a magnifying lens (Meiji Techno EMZ-13tr). High contrast digital images were obtained using reflected light with a dark background. The otoliths were placed with the outer face facing downwards, with the acoustic groove facing upwards and the rostrum pointing to the left.

Shape indexes

For the calculation of the shape indices (Tuset et al., 2003), the length of the otolith (OL), in mm, the weight of the otolith (OW), in mg, and the perimeter of the otolith (OPI), in mm, were recorded, in addition to the area of the otolith (OA), in mm^2, using the ImageJ software (Rasband, 1997) (Table 1).

Table 1. Otolith shape indices calculated from the morphometric measurements.

Shape indices	Formula
Shape factor	$(4\pi \times OA / OPI^2)$
Roundness	$(4OA) \times (\pi \times OL^2)$
Circularity	(OW^2 / OA)
Rectangularity	$(OA / OL \times OW)$
Ellipticity	$(OL - OW / OL + OW)$

OA: otolith area (mm^2); OPI: otolith perimeter (mm); OL: otolith length (mm); OW: otolith weight (mg).

Fourier elliptic analysis

Fourier coefficients were calculated using the SHAPE v.1.3 program (Iwata and Ukai, 2002). This program quantitatively evaluates biological forms based on Fourier elliptic descriptors and consists of three modules (ChainCode.exe; Chc2nef.exe; and PrintComp.exe), thus allowing the use of the data for other statistical analyses.

Elliptic Fourier analysis was applied to demarcate the shape (contour) via the “chain coding” algorithm, which represents an object as a closed two-dimensional curve, and applies a combination of harmonically related sine and cosine functions that are composed of four (a, b, c, and d) Fourier coefficients (Kuhl and Giardina, 1982). Based on the stabilization of the harmonic number curve, 20 harmonics were automatically calculated for each otolith, thus generating 80 coefficients per individual. The program standardized the size and orientation, and provided constant values for the first three coefficients, these being: a1 = 1, b1 = 0, c1 = 0. Each individual was therefore represented by 77 unique coefficients (Iwata and Ukai, 2002).

Statistical analysis

The measurements taken from the otoliths were submitted to descriptive analysis for calculations of frequency, mean, and standard deviation. Linear regressions were applied using the OL versus the shape indices to remove the allometric effect. When significant, we applied a correction using the Eq.1, proposed by Cardinale et al. (2004):

$$V_{aj} = V_i - b.OL \quad (1)$$

Where: V_{aj}: the adjusted variable; V_i: the variable analyzed; OL: the length of the otolith; b: its slope within the group.

Due to the high dimensionality of the descriptors (77 per individual), a principal component analysis (PCA) was applied to the variance-covariance matrix of elliptic Fourier coefficients generated for the specimens. To detect the significant eigenvalues, we plotted the percentage of the total explained variation of eigenvalues versus the proportion of expected variance estimated via the Broken-Stick model (MacArthur, 1957). The significant scores (PCs) were used as dependent variables in the subsequent analyses.

Multivariate analyses of variance with one factor (one-way MANOVA) using Pillai statistics were applied to test the hypothesis of there being an absence of differences in otolith shape for shape indices and Fourier PCAs. Then, canonical discriminant analyses were employed to find the combination of variables that detected intergroup differences in the MANOVA, which had the aim of graphically verifying the separation of groups and explaining the variations of the canonical axes. The success of classification into groups was assessed using jackknife cross-validation.

The assumption of multi-homogeneity of the variances within the groups (Anderson, 2006) was tested for each model using the Betadisper function, with Euclidean distance (Oksanen et al., 2016). When necessary, the outliers were removed to adjust the models. All statistical tests and graphical representations were performed using the R software (R Core Team, 2020), using the packages Candisc (Friendly and Fox, 2017), Vegan (Oksanen et al., 2016), mvOutlier (Reimann et al., 2012), and MASS (Ripley, 2011). The value of $p < 0.05$ was considered statistically significant for all analyses.

RESULTS

In the experimental fisheries, 74 pirarucu were captured, which had a weight variation between 4 to 102 kg (45.39 ± 26.14 kg) and measured from 40 to 213 cm in length (155.01 ± 36.02 cm). Out of the total number of pirarucu captured, 16 otoliths were extracted from fish from the Mamoré River, 45 were extracted from fish of the lower Guaporé River, and 13 from fish from the upper Guaporé River (Table 2).

Six significant main components (PCs) were determined, derived from the matrix of Fourier descriptors for the different areas, which accounted for 81.94% of the total variation. However, it was not possible to observe variations in the shape of the otoliths when the main components were superimposed (Fig. 2).

No significant differences in the shape of the otoliths were detected between the sites (A, B and C) via the MANOVA when the shape indices (Pillai = 0.196, $F_{(2, 136)} = 1.482$, $p = 0.152$) and the PCs (Pillai = 0.219, $F_{(2, 134)} = 1.378$, $p = 0.183$) were analyzed.

No evidence of relevant differences between groups was observed graphically for the two methods used (Fig. 3). For the shape index, the first canonical discriminant function (Can1) explained 57.4% of the total variance between the locations, while the second function (Can2) represented 40.79% (Fig. 3a). Similarly, the first discriminant function (Can1) of the Fourier series (PCs) contributed with 64.85%, and the second (Can2) with 35.14% (Fig. 3b).

The overall jackknife rating power of each model was similar, with 57.57% for the shape indices and 55.54% for the Fourier coefficients. The classification matrix was interpreted by observing the proportion of fish that are correctly classified in each group. Thus, the best-defined group was that of the stocks belonging to the lower Guaporé River, where it had the highest percentage of individuals classified, both for the shape indices (84.62%) and for the Fourier analysis (86.87%). On the other hand, the stocks belonging to the upper Mamoré River had a low classification with the same value for the shape indices and Fourier analysis (12.50%) (Table 3).

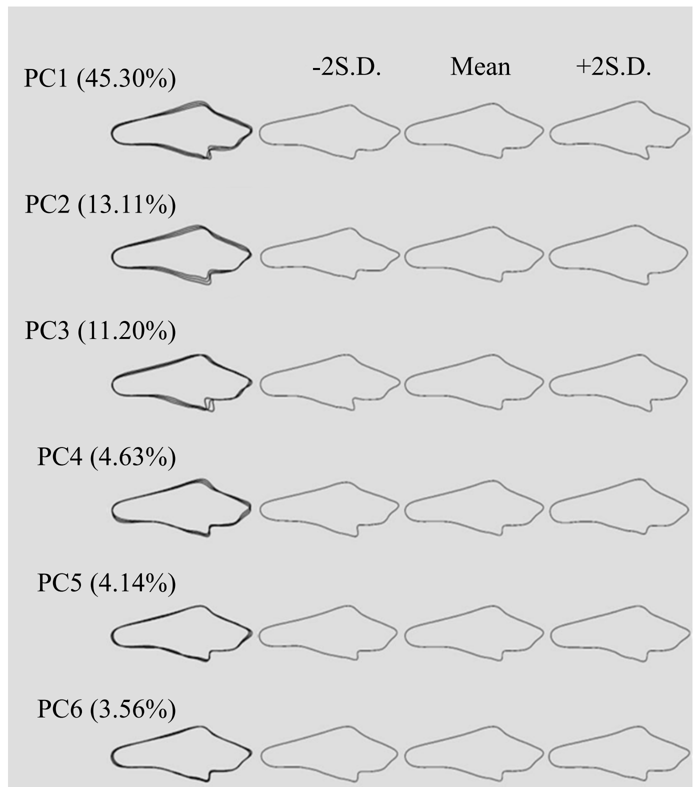

Figure 2. Variation in the shape (mean ± standard deviation) of *Arapaima gigas* sagitta otoliths explained by the first six major components (PCs).

Table 2. Descriptive analyses (mean ± standard deviation) of the measurements taken from the otoliths of the sample populations of pirarucu (*Arapaima gigas*).

Site	N	Length (mm)	Weight (g)	Area (mm²)	Perimeter (mm)
A	16	39.53 ± 5.91	1.75 ± 0.71	319.98 ± 92.03	99.09 ± 13.84
B	45	36.88 ± 6.89	1.32 ± 0.64	276.92 ± 89.47	93.07 ± 16.69
C	13	40.55 ± 3.54	1.65 ± 0.40	325.48 ± 50.27	107.19 ± 12.92

N: number of individuals sampled. A: Corte das Mercedes, upper Mamoré River; B: Extractive Reserve of the Cautário River, lower Guaporé River; C: Biological Reserve of the middle Guaporé River.

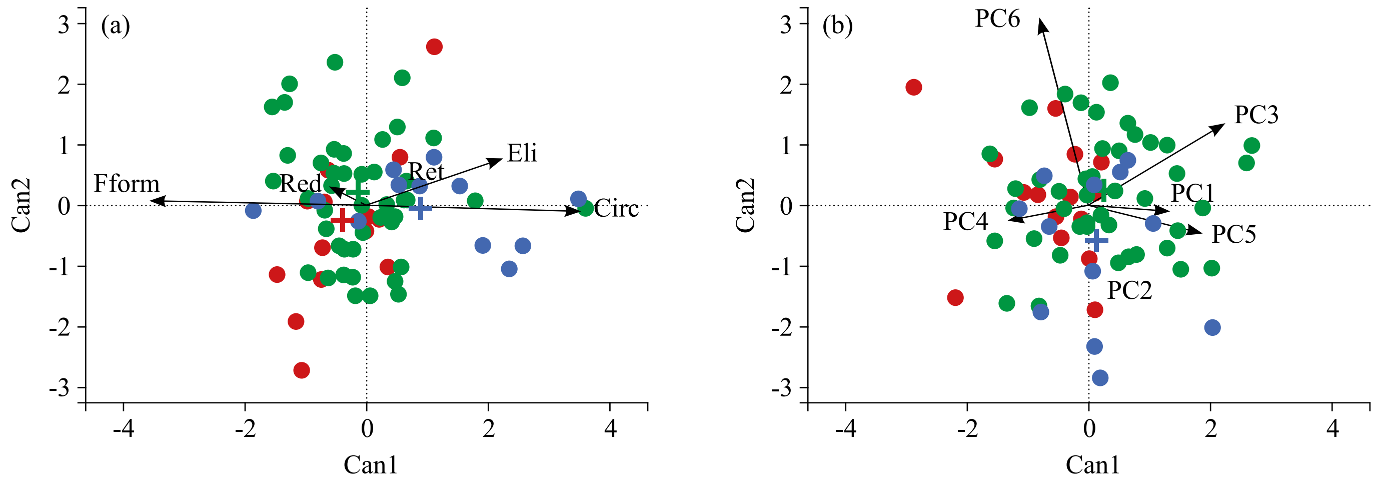

Figure 3. Canonical discriminant analysis using (a) morphometric indices and (b) elliptic Fourier coefficients measured at the three collection sites (site A: red, B: green, and C: blue). The vectors indicate the direction and intensity of the influence of the estimated characteristics: roundness (Red), rectangularity (Ret), ellipticity (Elip), circularity (Cir), and shape factor (Fform). PC1 to PC6 correspond to the significant scores of the principal component analysis performed on the Fourier matrix.

Table 3. Jackknife classification matrix for the analysis of the discriminant function of pirarucu (*Arapaima gigas*) individuals.

Site	Shape index			Fourier		
	A	B	C	A	B	C
A	2 (12.50%)	14	0	2 (12.50%)	12	2
B	3	33 (84.62%)	3	5	39 (86.67%)	1
C	1	7	3 (27.27%)	0	13	(0.00%)

A: Corte das Mercedes, upper Mamoré River; B: Extractive Reserve of the Cautário River, lower Guaporé River; C: Biological Reserve of the middle Guaporé River.

DISCUSSION

The absence of difference in the shape of the sagitta otoliths and the relatively moderate classification rates found in the present study suggest a single population of pirarucu *Arapaima gigas* along the sub-basins of the Mamoré and Guaporé rivers, which are the main tributaries of the right bank of the Madeira River. Our results corroborate those of Catâneo et al. (2022), who genetically characterized native and non-native *A. gigas* populations to elucidate basin invasion in the upper and lower Madeira River regions. Both sets of results show a clear separation between native and invasive populations, but they reveal a mixture of individuals from the upper and middle Madeira River. The population established in the upper Madeira River is the result of precursor events and was established from small groups of individuals introduced via escapes from farms in Peru (Catâneo et al., 2022).

The success of the introduction is due to the interaction between the characteristics of the species, the environmental changes caused by the implementation of two hydroelectric dams in the Madeira River, and the recurrent introductions from fish farms (Sousa et al., 2022). In this context, the increase in the pirarucu population and its records in the fishery landings of the middle and upper Madeira River regions have already consolidated the pirarucu as the most caught fish in this portion of the Madeira River in recent years (Doria et al., 2020).

In general, individuals from distinct environments are expected to show morphological differences in otoliths, even if they are genetically similar (Worthmann, 1979). According to Campana and Casselman (1993), compared to genetic factors, environmental factors are the most influential determinants of otolith shape. However, the studied regions have similar environmental characteristics since they are black-water rivers (acidic waters pH ≤ 4, low conductivity ≤ 8 µS•cm^{-1}, high transparency, between 1.3–2.9 m) (Junk, 1979; Sioli, 1984). In addition, the existence of connectivity between the areas allows the dispersion of the pirarucu throughout the different environments, which, despite the sedentary behavior of the pirarucu, some studies have reported large displacements for certain individuals (Crossa et al., 2003; Araripe et al., 2013).

The low variation observed in the contour of the otoliths may be due to the existence of a connection between the areas, thus allowing the homogenization of the population. In the lower Amazon, observations based on marking and recapture indicated a displacement of about 80 km for *A. gigas*, and months later they returned to their initial area, where they were marked (Crossa et al., 2003). Araripe et al. (2013) analyzed the dispersal capacity of the pirarucu and the structuring of its populations on distinct geographical scales and found the existence of high levels of genetic similarity between lakes separated by 25 km, a moderate gene flow between populations separated by large distances, and a low gene flow between stocks separated by distances of more than 1,300 km. This indicates that genetic differentiation between pirarucu populations occurs when they are separated by distances greater than 1,300 km, which may be mainly related to historical bottlenecks in population size.

Several studies on the natural populations of *A. gigas* have shown different levels of population structuring because of the geographical distance between the main rivers of the Amazon and the Araguaia-Tocantins basins (Hrbek et al., 2005; Araripe et al., 2013; Vitorino et al., 2015, 2017). A pioneering study in the Amazon basin showed a minimal substructure of pirarucu populations, which loosely fit an isolation by distance model (Hrbek et al., 2005). In a later study, two distinct groupings were detected; one composed mainly of fish from the lower Tocantins River and the lower Amazon River, and the other comprising predominantly individuals from the middle Amazon River (Araripe et al., 2013). Using ISSR markers, Vitorino et al. (2015) revealed that pirarucu populations of the Araguaia-Tocantins basin are structured and have low genetic diversity. In a more complete study, Vitorino et al. (2017) confirmed the population structure in the Araguaia-Tocantins basin; however, the results showed low levels of genetic diversity when compared to populations in the Amazon basin. More recently, a study involving the populations of the Araguaia-Tocantins and Amazon basin showed contrasts with these previous findings, since the authors identified a significant substructure in and between the basins (Torati et al., 2019).

Thus, understanding patterns of the morphometry of pirarucu otoliths at multiple geographic scales in the Amazon will require a greater sampling effort on a larger spatial scale, as well as sampling on a broader scale than just including other tributaries. In addition, multidisciplinary studies involving genetics, morphology, and ecology could provide useful guidance for the establishment of management units to contain the population increase of the pirarucu outside its area of natural occurrence.

CONCLUSION

In the present study, morphometric analyses in sagitta otoliths showed no differences for the sample populations of *A. gigas*, thus indicating a single population along the Mamoré and Guaporé rivers, which are the main tributaries of the right bank of the Madeira River. However, more complete studies are needed to elucidate the origins of invasions and create management measures to contain invasive populations in order to mitigate the possible damage that this species is causing to local fish assemblages.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

All dataset were generated or analyzed in the current study.

AUTHORS' CONTRIBUTION

Conceptualization: Mereles AM, Sousa RGC; **Investigation:** Sousa RGC, Freitas CEC; **Software:** Mereles AM; **Data curation:** Mereles AM; **Formal analysis:** Sousa RGC; **Resources:** Sousa RGC; Freitas CEC; **Validation:** Freitas CEC; **Supervision:** Sousa RGC; Freitas CEC; **Writing — original draft:** Mereles AM; **Writing — review & editing:** Mereles AM, Sousa RGC, Freitas CEC; **Final approval:** Sousa RGC.

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